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TITLE: A Genomic Approach to Identifying Novel Targets For Early Detection and Intervention of Prostate Cancer

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14. ABSTRACT

Early detection and intervention is key to a favorable prognosis in prostate cancer. Despite advances in the detection and treatment of prostate cancer, the mortality rate remains high. To improve survival, early detection and treatment strategies tailored to pre-invasive prostate cancer are required. We propose to catalogue genetic alterations associated with the developmental stages of disease for use as diagnostic tools and to identify the critical genes that drive the transformation of premalignant lesions to tumors for use as molecular targets for novel treatment design. The combination of laser capture microdissection (efficient isolation of specific cell types from hundreds of specimens) and SMAL DNA fingerprinting technology (high-through put analysis of genomic targets using minute quantities of DNA yielded from the microdissected cells) will facilitate systematic comparison of samples in various stages of disease development. By the end of this work, we will have identified a set of genetic loci (and genes) by virtue of their frequency of alteration in premalignant lesions and subsequently in low-grade tumors. We will have established a publicly accessible "genetic alterations in prostate cancer" database which catalogs somatic changes present in the various stages of cancer progression. Such information contribute to the fundamental understanding of prostate cancer pathogenesis.

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Early detection and intervention is key to a favorable prognosis in prostate cancer. Despite advances in the detection and treatment of prostate cancer, the mortality rate remains high. To improve survival, early detection and treatment strategies tailored to pre-invasive prostate cancer are required. We propose to catalogue genetic alterations associated with the developmental stages of disease for use as diagnostic tools and to identify the critical genes that drive the transformation of premalignant lesions to tumors for use as molecular targets for novel treatment design. The combination of laser capture microdissection (efficient isolation of specific cell types from hundreds of specimens) and SMAL DNA fingerprinting technology (high-through put analysis of genomic targets using minute quantities of DNA yielded from the microdissected cells) will facilitate systematic comparison of samples in various stages of disease development. By the end of this work, we will have identified a set of genetic loci (and genes) by virtue of their frequency of alteration in premalignant lesions and subsequently in low-grade tumors. We will have established a publicly accessible "genetic alterations in prostate cancer" database which catalogs somatic changes present in the various stages of cancer progression. Such information contribute to the fundamental understanding of prostate cancer pathogenesis.

Annual Report for Award Number DAMD17-01-1-0028

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Principle Investigator: Wan L. Lam, Ph.D.
Co-Investigator: Juergen R. Vielkind, Ph.D.

Author: Wan L. Lam

1. Introduction

Accumulation of key genetic alterations is though to be the underlying mechanism that drive the development of prostate cancer, and such changes have not been well defined. The goal of this project is to apply high-resolution DNA fingerprinting technology to identify key genetic alterations associated with the progression stages of prostate cancer.

1.1. Background

Early detection and intervention is key to a favorable prognosis in prostate cancer (CaP), as in many types of cancers. Disease progression is thought to be driven by cumulative genetic alterations affecting a small number of genes (see Kinzler and Vogelstein, 1996). Identification of these genes would provide novel targets for diagnosis and intervention. Despite many efforts these genes have not yet been identified. One possibility to discover these genes is by identifying genetic alterations that parallel the histopathological progression stages in prostate cancer. This can only be achieved by a genome-wide screen for alterations.

CaP consists of a mixture of normal epithelial cells, stromal cells, benign hyperplastic cells, PIN, and various clones of invasive carcinoma. To date, due to the difficulty in obtaining sufficient material from the small early progression stages and the lack of analytical methods applicable for genome-wide scanning of minute clinical specimens, much of our knowledge on genetic changes in CaP has been derived from analyses of advanced tumors and cell lines, which are characterized by complex genetic changes many of which may be random, due to generalized genetic instability, rather than being etiologic. Thus, genetic alterations relevant to disease progression may be more easily identified by examining stages earlier in the development of a tumor, before the accumulation of randomly altered changes occurs. We therefore focus on identifying genetic changes in premalignant lesions which subsequently appear in the early invasive stages of CaP. "Normal epithelium" versus "PIN" comparison will reveal the spectrum of genetic changes in the early stages of CaP development, while the "PIN" to "tumor" comparison will identify the subset of changes critical to disease progression.

The objective of this proposal is to identify the progressive genetic alterations that cause normal prostate epithelial cells to transform into precursor prostatic intraepithelial neoplasia and invasive cancer cells of low Gleason grade. This will be achieved by systematically

comparing high density DNA fingerprints of microdissected samples in various stages of disease development.

Over a 3 year period, we proposed to compare DNA samples extracted from normal cells, from precancerous cells and from tumor cells in a variety of patient biopsies, in order to identify key genetic changes in the stages of disease development. Our results in is described here in relation to the original *Statement of Work.*

2. Body

Much of the details of this project have been described in previous Annual Reports. This document represents the assembly of previous reports, update of new findings and a final summary of the work completed for Award DAMD17-01-1-0028, 2001-2004.

In this project, we have proposed to use laser capture microdissection to selectively isolate pure cell populations representing normal epithelium, benign hyperplasia, high grade PIN and invasive cells of low Gleason grades 1-3. We have couple this cell isolation approach with SMAL-PCR DNA fingerprinting technology in order to analyze these various CaP progression stages at thousands of randomly distributed genetic loci for frequent alterations.

The specific aims are:

1. To generate SMAL DNA fingerprints from normal epithelium, PIN and invasive carcinomas of early stages.
2. To identify recurring alterations that are present in the early progression stages.
3. To establish an expandable database of recurring changes for each stage of disease development.
4. To assign recurring alterations to specific chromosomal regions.
5. To identify candidate genes in the mapped regions for future mutation scanning.
6. To begin validation of candidate genes.

Aims 1-4 started in Year 1 of the project extending into Years 2 and 3, while aims 5 and 6 are Year 2-3 activities. Comparing our accomplishments against the original Statement of Work is presented in the Reportable Outcomes section below.

2.1. Experimental design and methodology

There is no deviation from the design proposed in the original research plan.
As mentioned in the previous annual reports, the methodologies were described in detail in the original proposal. This is a brief description of the two key technologies established and used in this project.

Laser Capture Microdissection.

To overcome tissue heterogeneity in the prostate biopsy specimens, each sample is evaluated histopathologically and then subjected to microdissection. As described in previous Annual Reports serial 5mm sections are prepared and each placed in the centre of an uncoated glass slide facilitating laser capture of the target cells. Slide 1 to 5 are stained with toludine blue. Slides 1-4 will be kept desiccated until used while slide #5 is be coverslipped and used as the reference slide for pathological evaluation. The remaining slides are kept in reserve for verification of experimental result and/or used if not enough cells can be microdissected from slides 1-4.

Under direct microscopic observation, a vial cap, which carries a thermoplastic film on its undersurface, is placed over sections from paraffin-embedded or frozen biopsy material. A laser is then aimed over the desired cells in the sections. Upon activation of the laser, the targeted cells are selectively adhered onto the film and can be removed for further analysis (for review of LCM see: Pappalardo et al., 1998; Simone et al., 1998). DNA is extracted from the captured cells. DNA concentrations are determined by quantitative PCR comparing against a standard curve generated from the amplification of known DNA quantities of genomic DNA.

SMAL-PCR DNA fingerprinting.

This technique is designed for systematic high density scanning for alterations in cancer cells. Due to the minute quantities of DNA available in microdissected samples, we have modified the conventional Arbitrarily Primed-PCR (AP-PCR) DNA fingerprinting (Peinado et al., 1992; Ionov et al., 1993) technology for analyzing microdissected cells. We have named the new technique Scanning of Microdissected Archival Lesions, SMAL-PCR. As in AP-PCR, SMAL-PCR utilizes multiples of short, arbitrary primer pairs of 10 nucleotides to simultaneously amplify a large number of targets randomly distributed throughout the genome. The resulting PCR fragments are separated on acrylamide gels allowing the identification of polymorphic genetic alterations between, e.g. normal and cancerous tissue.

This technique has yielded much more genetic information than that provided by the conventional assays for detecting chromosomal alterations. We have shown that highly reproducible DNA fingerprints could be generated from < 2 ng of DNA (300 cell equivalents) extracted from cells of various archived tumor specimens (Siwoski et al 2002).

2.2. Progress in Specific Aims

Aim 1: Generate SMAL DNA fingerprints from normal epithelium, PIN and tumors

Procurement of archival tissue:
Tissue acquisition. A large number (hundreds) of paraffin embedded CaP biopsies have been evaluated histopathologically in order to identify specimens containing pre-cancerous and cancerous lesions (cells) suitable for microdissection. Under the supervision of two pathologist, areas of normal epithelium, PIN and invasive carcinoma were identified (Figure 1).

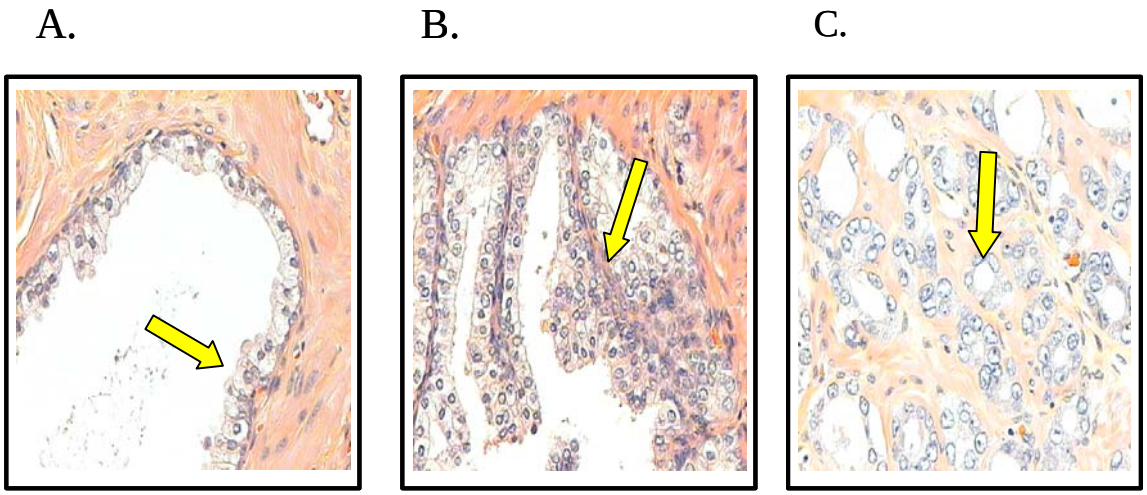

Figure 1. Partial images of a cross-section through a formalin-fixed paraffin-embedded whole-mount prostate gland showing (yellow arrows) the histology of (A) normal, (B) prostatic intraepithelial neoplasia (PIN) and (C) early carcinoma cells of Gleason grade 3.

LCM, DNA extraction and SMAL fingerprinting:

The optimization of LCM was described in detail in the previous report and will not be addressed here, except that one example is provided in Figure 2.

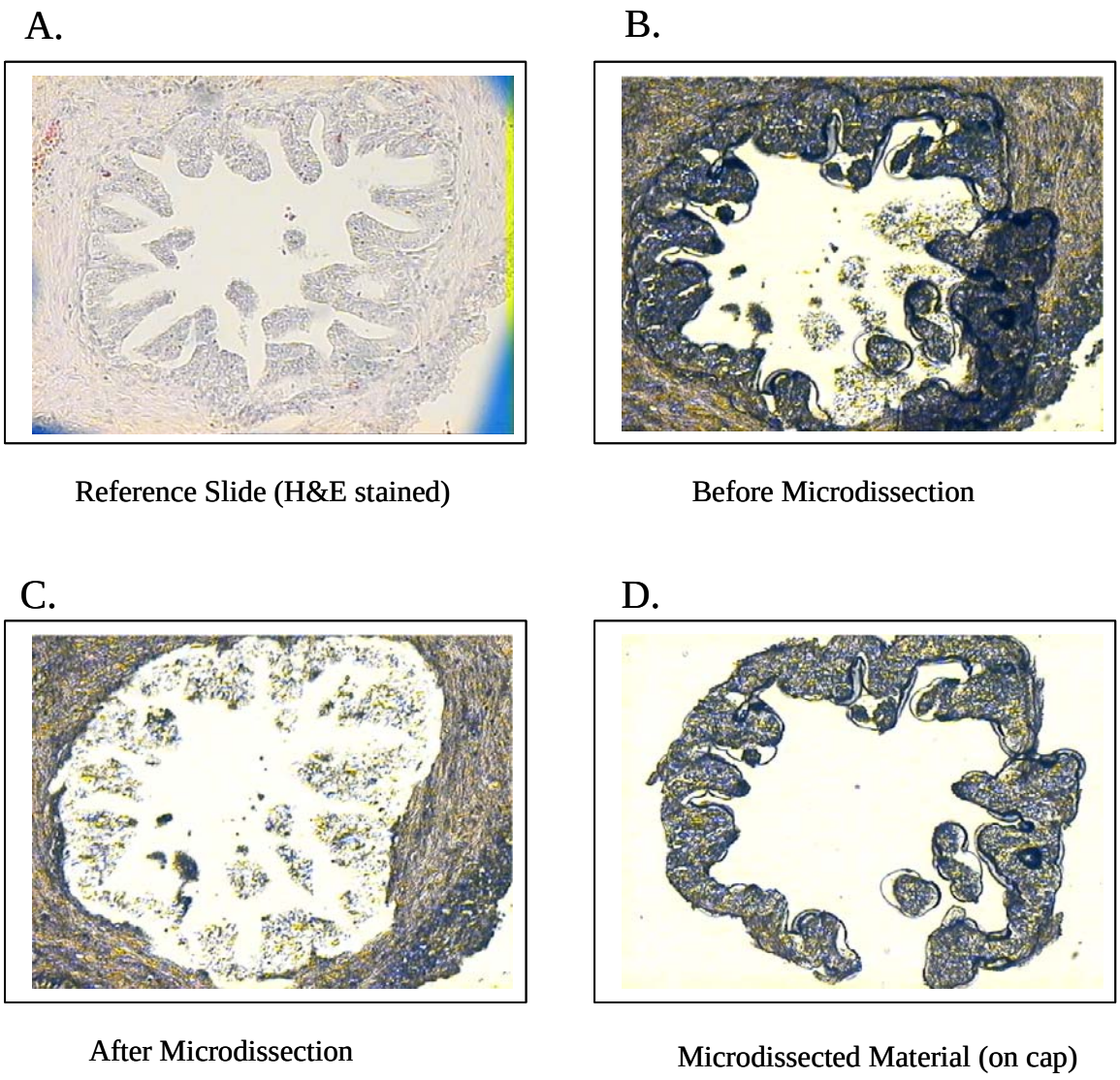

Figure 2. Microdissection of PIN from toluidine blue stained sections of formalin-fixed paraffin-embedded prostate archival biopsies by Laser Capture Microdissection (LCM).

Optimization of DNA extraction protocol achieved in Year 2 increased DNA yield for SMAL PCR experiments. Figure 3 shows the effects of various digestion buffers on DNA extraction and yield and Figure 4 shows the evaluation of DNA yield using a multiplex PCR approach.

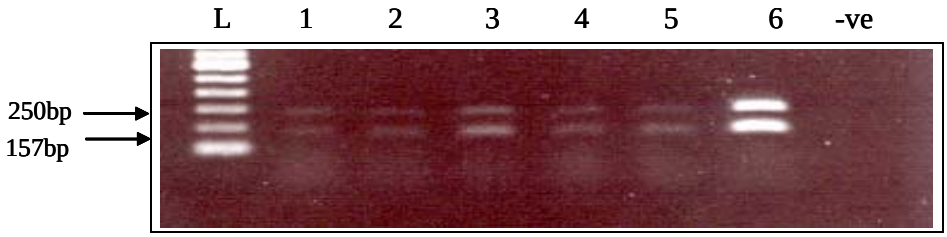

Figure 3. Effects of various digestion buffers on DNA extraction and yield.
Lane 1 = 10mM Tris/1mM EDTA (pH 8.0), 1% Tween 20; Lane 2 = 50mM Tris/1mM EDTA (pH 8.0), 1% Tween 20; Lane 3 = 50mM Tris/1mM EDTA (pH 7.5), 1% Tween 20; Lane 4 = 10mM Tris/2mM EDTA (pH 8.0), 1% Tween 20; Lane 5 = 10mM Tris/2mM EDTA (pH 8.0), 0.5% SDS; Lane 6 = 10mM Tris/1mM EDTA (pH 8.0), 0.5% SDS and 50mM NaCl [+0.1% proteinase K digestion overnight at 42^{o}C]

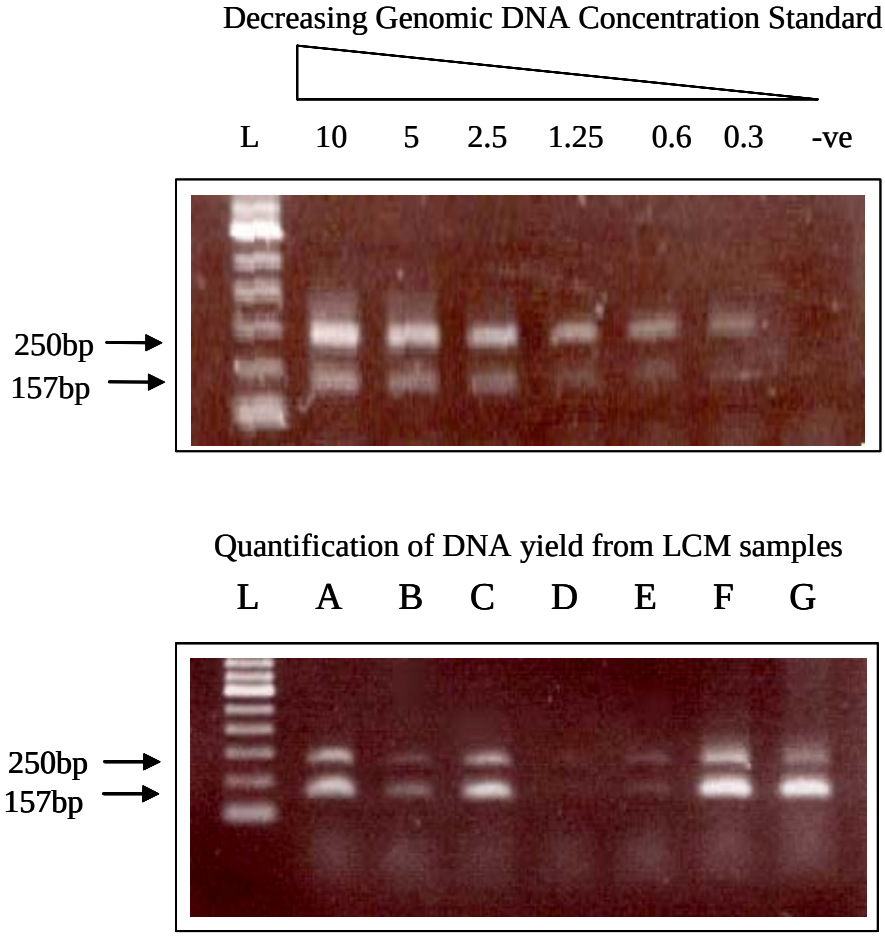

Figure 4. Assessing DNA quantity and quality of microdissected prostate samples by multiplex PCR. PCR amplification (30 cycles) of 250 bp and 157 bp LEN gene fragment of microdissected samples (A-G) of unknown quantity and quality compared to amplification of a decreasing genomic DNA concentration standard.

Genomic DNA was extracted from 75 laser capture microdissected prostate samples (selected from the hundreds evaluated); they comprised of 28 samples of normal cells, 15 samples representing cells from prostatic intraepithelial neoplasia (PIN) and >30 samples of early invasive carcinoma of varying degrees (Gleason grades 2 to 4) from 28 formalin-fixed, paraffin-embedded radical prostatectomy specimens. Sections from these prostatectomy specimens were microdissected by laser capture microdissection (LCM) to obtain matched samples of normal, prostatic intraepithelial neoplasia (PIN) and early invasive carcinoma cells (Gleason grades 3). Genomic DNA was extracted from these cell samples and pairs of normal and tumor DNA samples (at 2 nanogram each) were subjected to SMAL-PCR DNA fingerprinting that scans microdissected archival lesions for genetic alterations.

Aim 2: Identify recurring alterations that are present in PINs and tumors

Comparison of DNA fingerprints between normal cells, PIN and/or tumors from individual patients revealed genetic alterations (gain or loss of PCR signal). Figure 5 is a schematic diagram of this technology.

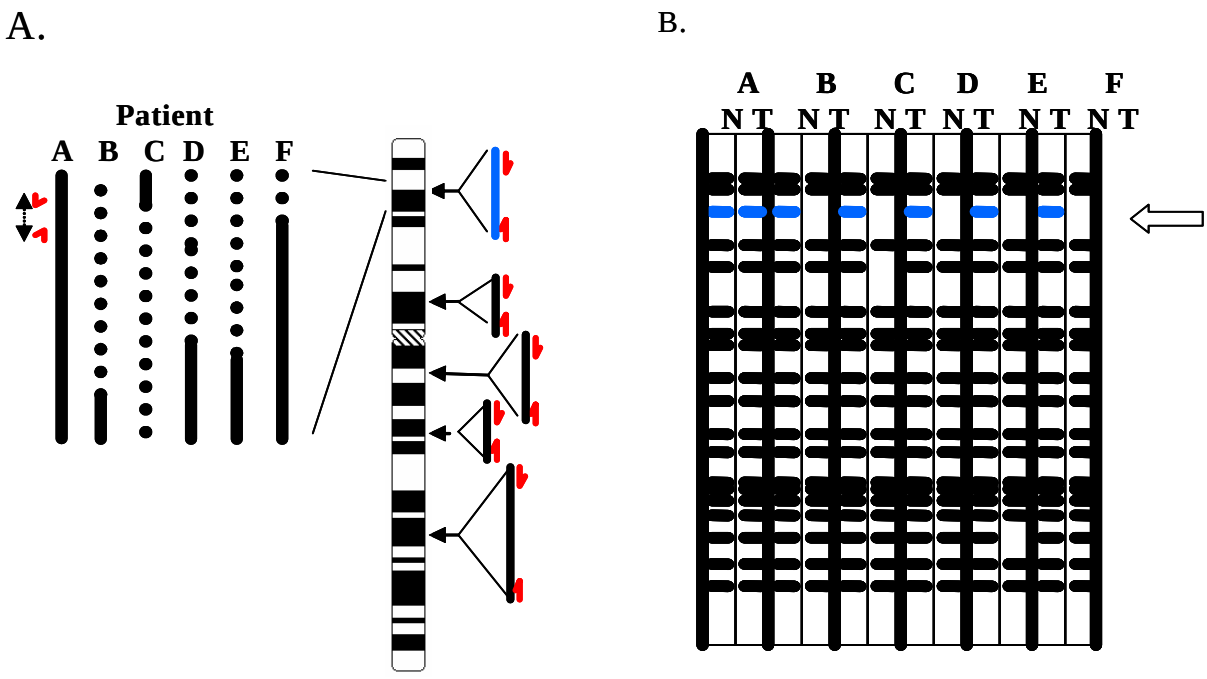

Figure 5. Principle of SMAL-PCR DNA fingerprinting: (A) A single arbitrary primer pair (red arrows) binds to multiple targets and is used to amplify genomic DNA on specific regions of a chromosome. Alterations in DNA in patients B to F do not allow primer binding (blue bar) and thus (B) result in loss of a polymorphic fragment in patients B to F.

Comparison amongst a panel of patients revealed recurring genetic alterations (for example, Figure 6). The criterion for the initial screen is that an alteration has to occur more than once in >10% of cases. More than 25 recurring polymorphic fragments have been cloned, sequenced. Localization of these sequences to specific chromosome regions (see Aim 5 below) have been performed in Year 2 of this project as scheduled.

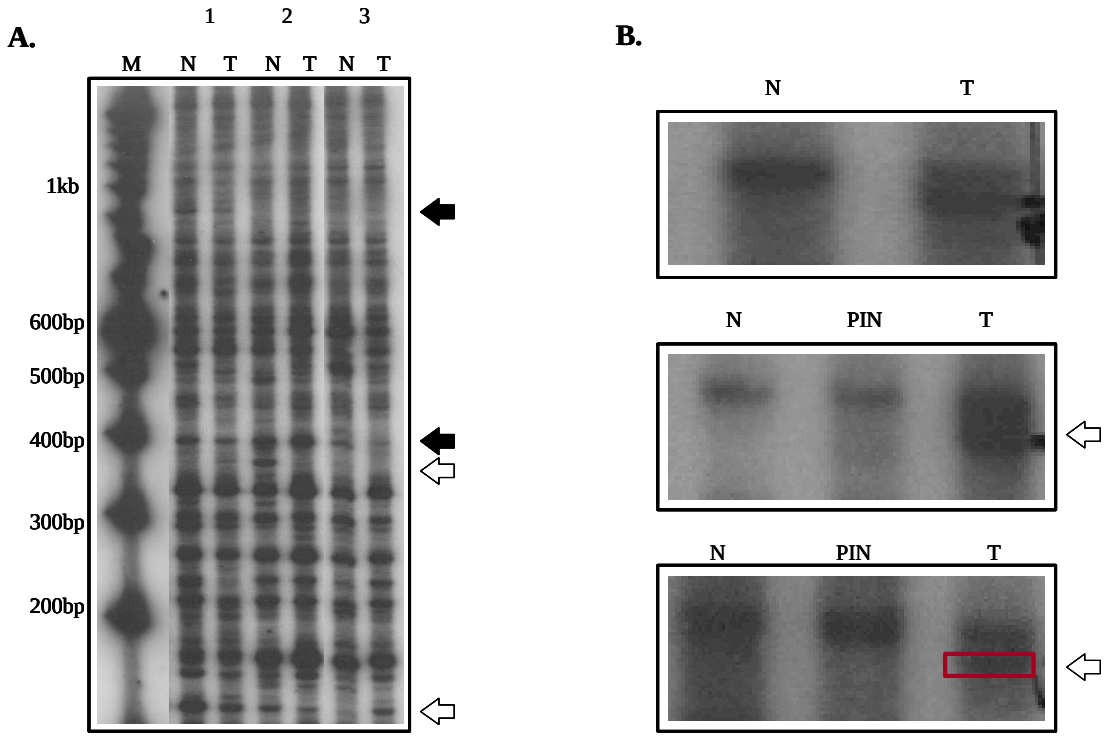

Figure 6. (A) SMAL fingerprints from paired prostate normal (N) and early tumor (T) cells from 3 CaP patients. (B) An example of a 250bp recurrent gain in tumor samples, identified in 3 of 14 patients screened. Closed arrows indicate examples of polymorphisms between patients and open arrows indicate differences between normal and tumor samples.

Selected DNA fragment, such as those show in Figure 6 above, were excised from the dried gel guided by the autoradiograph. Re-amplification yielded sufficient quantities of material for cloning into plasmid vectors. Figure 7 the raw data of re-amplification for two of the recurring SMAL-PCR fragments (see next page). Colony PCR is used to confirm the cloning of the correct size fragments (Figure 8).

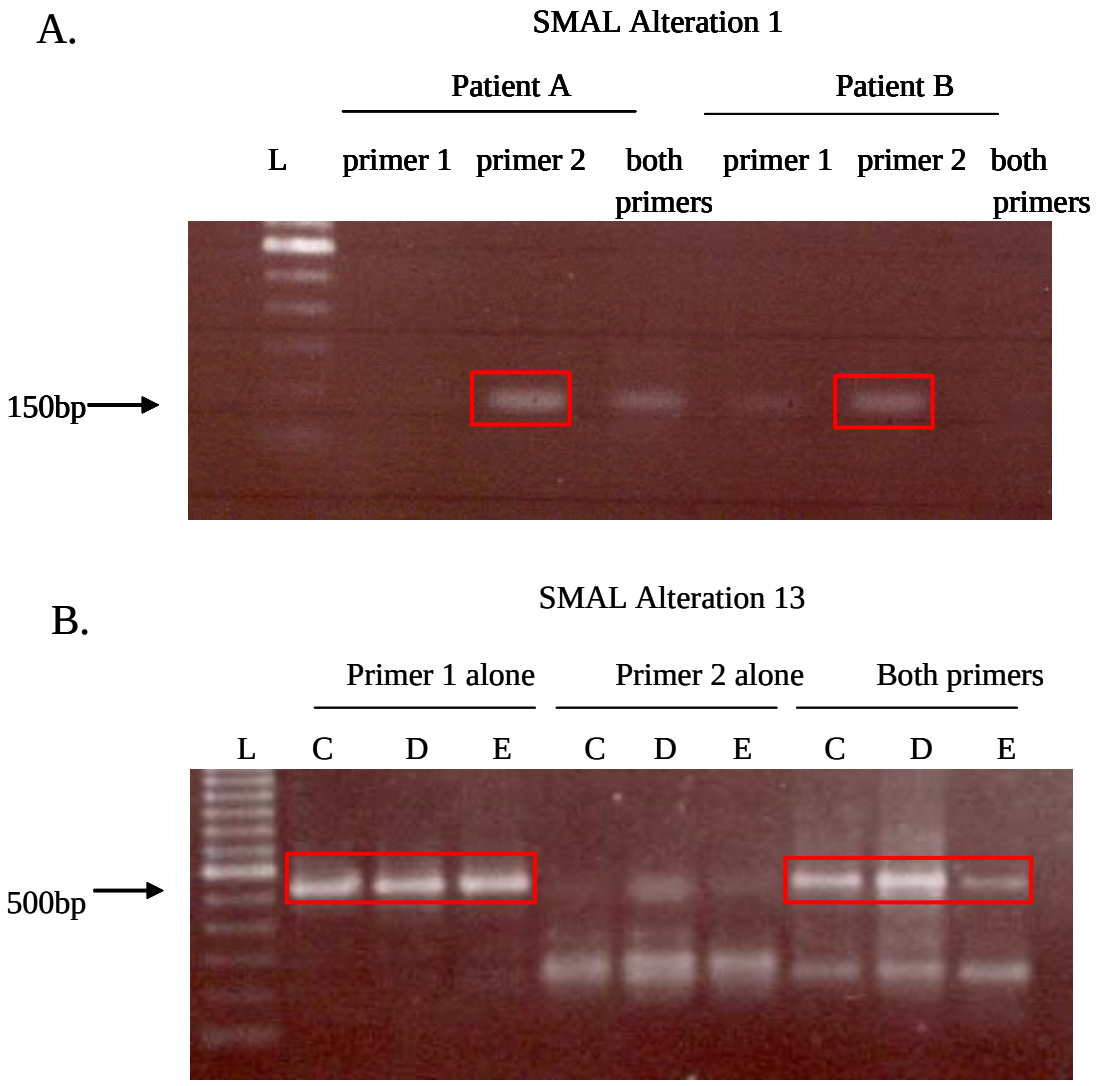

Figure 7. 1% Agarose gel of re-amplification of (A) SMAL alteration 1 (150bp) in two patients A and B; and (B) alteration 13 (500 bp) in three patients C, D and E with primer 1 alone, primer 2 alone and both primers combined.

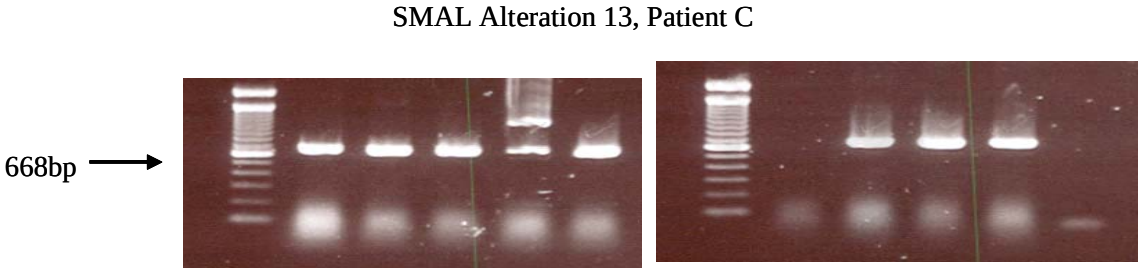

Figure 8. Colony PCR of patient C in alteration 13 (primers 1 alone); 8 of 9 transformed clones were confirmed to contain the expected size (500 bp insert + 168 bp vector = 668 bp).

The identity of each clone is determined by DNA sequencing (Figure 9).

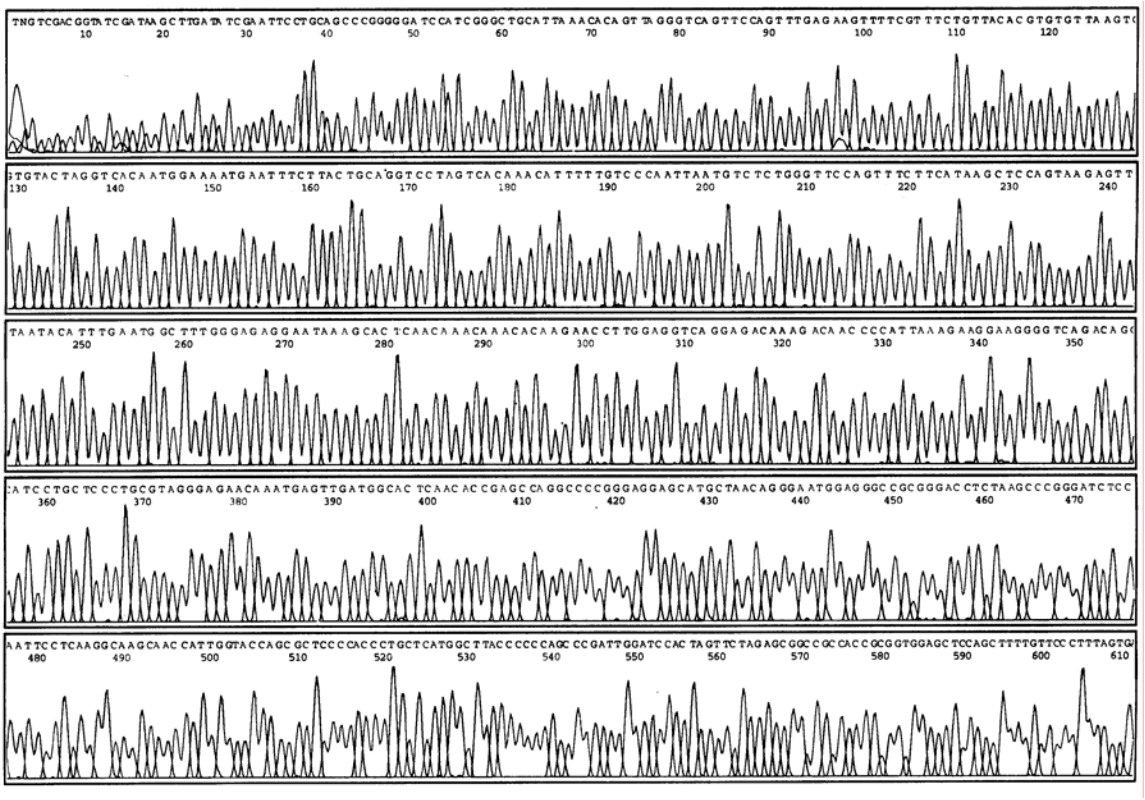

Figure 9. DNA sequence of alteration 13. Sequence determined at the Nucleotide Acid Protein Services (NAPS) Unit at the University of British Columbia.

Aim 3: To establish an expandable database of recurring genetic changes

A database of recurring genetic alterations has been established. Information on recurring changes, such as frequency of occurrence, histopathological stage of sample, sequence of DNA fragment have been entered into this database. The output from this database is summarized in Figure 10 and Table 1 below.

Aim 4: To assign recurring alterations to specific chromosomal regions

The sequence of each SMAL-PCR alteration is use to identify specific human bacterial artificial chromosome (BAC) clones containing the sequence. In turn, the location of the BAC clone in the human genome informs the chromosomal location of the recurring genetic alteration.

Genetic alterations were discovered at the chromosomal regions listed in Table 1. Loci at 2p23.1, 8q22.3 and 18q21.31 were unique to PIN while changes at 3p26.3, 4q35.2, 8q24.13,

11p13, and 16q12.2 were detected only in cancer cells; alterations at 1p32.1, 1q44, 5q23.2, 9q22.32 and at 12p12.1 were found in both stages (Figure 10).

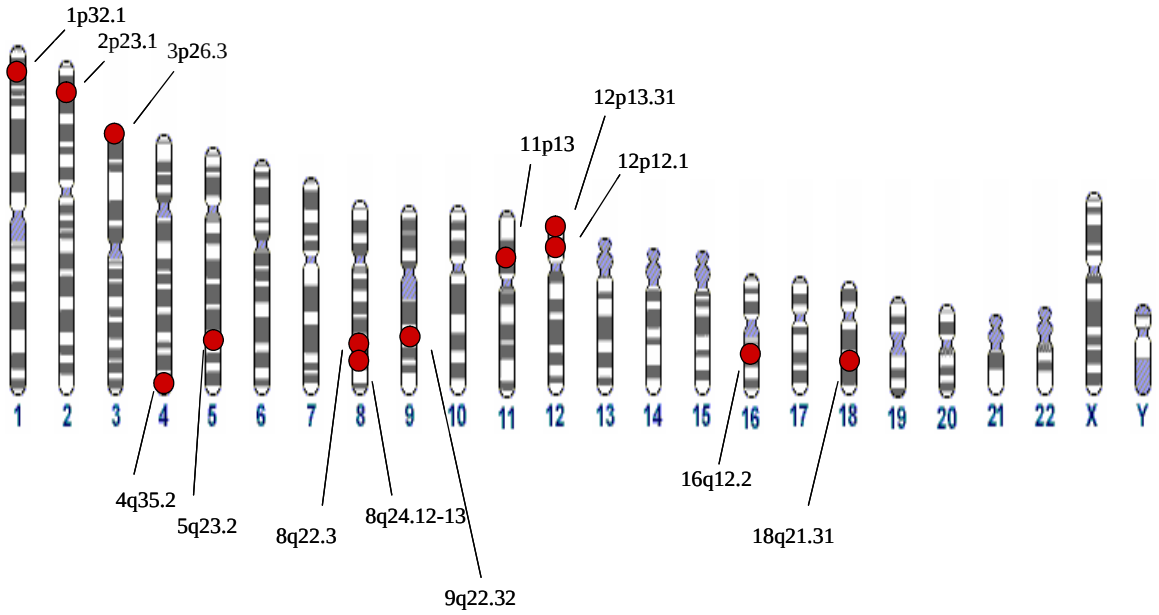

Figure 10. Illustration of the chromosome regions that display instability in CaP as identifed by SMAL-PCR.

A detailed summary of the recurrent alterations identified is shown in Table 1. Chromosomal location, assigned patient number, histological stage of lesions, as well as information on the identified fragments are listed.

In addition, DNA probes generated from selected BACs have been used in fluorescent in situ hybridization experiments on tissue sections from radical prostatectomy specimens to validate the alterations detected by SMAL.

Table 1. Summary of recurrent alterations identified.

	Chromosome Localization	Assigned Patient No	Stage at which change is seen		Gain or Loss of Polymorphic Fragment	Frequency	BAC clone
			PIN	Early Inv Ca			
1	18 (18q21.31)	K2	156bp		gain	2/28	RP11-845C23
		K11					
2	8q22.3	K3	184bp		gain	3/28	NM_018544
		K11					
		K15					
3	no recurrent pattern	K11	210bp		gain	2/28	
		K15					
4	no recurrent pattern	K3		240bp	loss	3/28	
		K7					
		K15	240bp				
5	no match	K4	270bp	270bp	loss	3/28	
		K7					
		K15					
6	4q35.2	K5		275bp	loss	3/28	RP11-11N5
		K8					
		K4					
7	5q23.2	K8		285bp	gain	2/28	CTC-369A16
		K16					
8	5q23.2	K7		272bp	loss	2/28	CTC-369A16
		K11	272bp				
9	3p26.3 / 12p13.31	K2		320bp	gain	3/28	RP11-024C23
		K5					
		K8					
10	no recurrent pattern	K3	400bp	400bp	loss	3/28	
		K15					
		K16					
11	12p12.1	K4	420bp	420bp	loss	2/28	RP11-16A24
		K15					
12	1p32.1	K11	470bp	470bp	loss	4/28	RP4-695L18 and RP11-436K8
		K12					
		K15					
		K16					
13	11p13	K2		495bp	loss	3/28	RP11-85M6
		K4					
		K15					
14	16q12.2	K3		635bp	loss	4/28	CTD-2545G24 and RP11-2D4 and RP11-
		K4					
15	8q24.12-13	K6		561bp	gain	4/28	RP11-682G1 and RP11-711B6
		K7					
		K8					
		K11					
16	4+4 mapped to 2p23.1	K1	203bp		gain	3/28	RP11-541A15
		K11					
		K15					
17	no recurrent pattern	R5		200bp	loss	2/28	
		R22					
18	no recurrent pattern	K24	240bp		gain	3/28	
		R5					
		R22					
19	failed reamp	K17		245bp	loss	2/28	
		R6					
20	9q22.32	R6	290bp	290bp	loss	3/28	RP11-569G13
		R5					
		R22					
21	failed reamp	R6		350bp	gain	2/28	
		VGH6	350bp				
22	11p13	R3		500bp	loss	2/28	RP11-85M6
		R6					
23	failed reamp	VGH5		550bp	loss	3/28	
		VGH6	550bp				
24	failed reamp	K21		680bp	loss	3/28	
		R6					
		R5					

Aim 5: To identify candidate genes in the mapped regions for future mutation scanning

We have identified candidate genes in all of the chromosomal regions of alteration, described in Aim 4 above, by computational searches of all known cDNA, EST and microarray and SAGE expression data bases. Our studies identified several altered chromosomal regions of which includes 1p/q, 4q, 5q, 8q22/24, 10q, 11p 16q and 18q that have been previously reported in the literature. More importantly, novel sites which mapped to chromosomes 2p23.1, 3p26.3, 3q22.3, 4q22.3, 5q23.2, 9q21.3, 9q22.32, 12p12.1, 13q12.2 and 16q12.2 were also found. Mapping these regions within a 2Mbp range was undertaken and some interesting genes were identified. For example, 3p26 harbors a major CaP predisposition locus, those that mapped to 1q44 and 11p13 contain oncogenes, *AKT3* and *LMO2*, respectively, while an alteration that mapped to 4q35.21 contains the FAT tumour suppressor gene.

Aim 6: To begin validation of candidate genes

In the original proposal, we anticipated to begin validation of candidate genes (the best two genes) near the end of the project in Year 3. Although only preliminary analysis of candidate genes was proposed due to the time frame of the project, follow up studies and new collaborations has validated the significance of one such gene *LMO2* (Ma et al 2007).

3. Key Research Accomplishments

1. Procurement of a panel of histopathologically graded prostate specimens and the identification of >75 lesions suitable for microdissection.

2. Laser capture microdissection of >75 samples. The LCM technology is well established in Dr. Vielkind's (co-applicant) laboratory. In addition to the Arcturus PixCell II laser capture microdissection device described in a previous annual report, two additional laser assisted microdissection devices have been established based on funds provided by other granting agencies. These equipments have allowed us to tailor dissection strategy for specific tissues.

3. The isolation of pure cell populations have supported the proposed DNA fingerprinting effort, and the LCM protocol established has enabled cell isolation in related projects supported by other funding agencies. This has yielded gene expression profiles by serial analysis of gene expression (SAGE) and proteomic studies that led to the discovery of the association of Growth Differentiation Factor 15 (GDF15) to prostate carcinogenesis (Cheung et al 2004).

4. Identification of >24 recurring genetic alterations in PIN and/or prostatic carcinoma. We have observed that some of the alterations identified map to chromosomal regions previously unknown to be involved in prostate cancer development.

5. Discovery of candidate genes. As mentioned in Aim 5 above, our studies identified several known and novel altered chromosomal regions. Mapping these regions identified candidate genes, for example, AKT3 and LMO2, and the FAT tumor suppressor gene. Follow up experiments performed after the completion of this project by Dr. Vielkind (co-applicant) and collaborators has suggested the significance of LMO2 expression in the progression of prostate cancer (Ma et al 2007).

6. Work on laser capture microdissection was presented at 4 conferences during the project.

Ma S, Bainbridge CT, Webber D,Sutcliffee M, Hui M, Adomat H, Lam W, Vielkind JR A concerted genomic and proteomic approach to understanding the early stages of prostate carcinogenesis. American Association for Cancer Research, Washington D.C. (July 2003)

Stephanie Ma, Hans Adomat, Terry Bainbridge, Doug Webber, Wan Lam, Juergen Vielkind. A concerted genomic and proteomic approach to understand the early steps in the genesis of prostate cancer. American Association for Cancer Research, San Francisco, CA (April 2002)

Stephanie Ma, Hans Adomat, Terry Bainbridge, Doug Webber, Wan Lam, Juergen Vielkind. A concerted genomic and proteomic approach to understand the early steps in prostate carcinogenesis. British Columbia Cancer Agency Conference, Vancouver, Canada (Nov 2001)

Stephanie Ma, Hans Adomat, Terry Bainbridge, Doug Webber, Wan Lam, Juergen Vielkind (2001) A proteomic approach to understand the onset and early progression of prostate cancer using Surface Enhanced Laser Desorption Ionization (SELDI) proteinchip array. Laser Capture Microdissection Symposium, NIH, Washington, DC (July 2001)

7. The following Abstracts were published (2001-2004) in the proceedings of international conferences:

Ma S, Siwoski A, Bainbridge CT, Woocock B, Lam WL, Vielkind JR (2001) Optimization for integrity and yield of DNA extraction from laser capture microdissected cells from archival biopsies. American Assoc. for Cancer Research Proceedings 42: 532.

Ma S, Adomat H, Bainbridge CT, Webber D, Lam W, Vielkind J (2002) A concerted genomic and proteomiv approach to understanding the early steps in the genesis of prostate cancer. American Assoc. for Cancer Research Proceedings 43: 683.

Ma S, Bainbridge CT, Webber D,Sutcliffee M, Hui M, Adomat H, Lam W, Vielkind JR (2003) A concerted genomic and proteomic approach to understanding the early stages of prostate carcinogenesis. American Assoc. for Cancer Research Proceedings 44: 739.

Ma S, Chan KW, Woolcock B, Bainbridge T, Webber D, Lam W, Wong KY, Guan XY, Vielkind J (2007) DNA fingerprinting tags novel altered chromosomal regions and identifies the involvement of LMO2 in the progression of prostate cancer. 98th Annual American Assoc. Cancer Research Conference Proceedings, #4441

(This is based on a follow up study after the completion of project.)

and local conferences:

Garnis C, Ishkanian A, Ralph S, Kasteel K, Rosin M, Lam W (2001) Identification of Genetic Alterations in Cancer Using RAPD-PCR and cDNA Microarrays. 2nd Annual Pathology Day Gala, Vancouver, BC, Canada.

Ma S, Adomat H, Bainbridge T, Webber D, Lam W, Vielkind J (2001) A concerted genomic and proteomic approach to understand the early steps in prostate carcinogenesis. 2001 BC Cancer Annual Conference, Vancouver, BC, Canada.

Ling V, Marra M, Eaves C, Herst S, Jones S, Lam S, Lam W, MacAulay C, Rosin M, Sadar M, Vielkind.J (2001) CANCER GENOMICS: A multi-disciplinary approach to the large-scale high-throughput identification of genes involved in early stage cancers. 2001 BC Cancer Annual Conference, Vancouver, BC, Canada.

Ma S, Ge Y, Woolcock B, Bainbridge T, Webber D, Sutcliffe M, Lam W, Vielkind J (2003) Genome-wide detection of genetic changes in early prostate carcinogenesis. 2003 BC Cancer Annual Conference.

8. The following peer-reviewed journal publications were made possible due to the foundation of information and know-how established by this project.

Cheung PK, Woolcock B, Adomat H, Sutcliffe M, Bainbridge TC, Jones EC, Webber D, Kinahan T, Sadar M, Gleave ME, Vielkind J. Protein profiling of microdissected prostate tissue links growth differentiation factor 15 to prostate carcinogenesis. Cancer Research **64**: 5929-3. 2004.

Ma S, Guan XY, Beh PS, Wong KY, Chan YP, Yuen HF, Vielkind J, Chan KW. The significance of LMO2 expression in the progression of prostate cancer. J Pathology **211**: 278-85, 2007.

Noorali S, Kurita T, Woolcock B, de Algara TR, Lo M, Paralkar V, Hoodless P, Vielkind J. Dynamics of expression of growth differentiation factor 15 in normal and PIN development in the mouse. Differentiation **75**: 325-36, 2007.

Siwoski A, Ishkanian A, Garnis C, Zhang L, Rosin M, Lam WL. An efficient method for the assessment of DNA quality of archival microdissected specimens. Modern Pathology **15**: 889-92, 2002.

4. Reportable Outcomes

The scientific accomplishments for Year 2 is listed in section 3 above. This section measures our progress (reportable outcomes) against the original Statement of Work (Task 1-6).

Task 1. **To generate DNA fingerprints from normal epithelium, PIN and early invasive carcinomas (months 1-30)**

- case selection and pathology review of 300 cases (months 1-30)
- laser capture microdissection of 150 samples for each stage (months 1-30)
- DNA preparation, quantitation and quality control (months 1-30)
- generate 90,000 DNA fingerprints using 20 primer pairs for each of the 450 samples (months 4-30)
- collect and process fingerprint images for analysis (months 4-30)

Accomplishment: This *Task* spaned Year 1 and 2. For month 1-24, the following reportable outcomes have been achieved.

- Hundreds of cases have been reviewed by collaborating pathologists to identify 75 suitable for microdissection
- >75 samples (ie. Normal, PIN, cancer) were microdissected
- DNA was extracted from all samples
- Hundreds of SMAL-PCR profiles were generated using 1 primer pair; on average, 30 fingerprint bands are produced per profile giving an estimate of 10,000 fingerprint bands.
- DNA fingerprinting experiments were completed in month 25-30.

Task 2. **To identify recurring alterations that are present in the stages (months 4-30)**

- compare pairs of fingerprint images within each histopathological stage to identify recurring alterations (months 4-36)

Accomplishment: At the completion of this task, a total of 24 recurrent changes were identified (66 separate bands, originating from the 24 recurrent changes, were isolated). After cloning and sequencing, 20 recurring genetic alterations have been identified.

Task 3. **To establish an expandable database of recurring changes for each stage of disease development (months 4-30)**

- establish and format database
- data entry as information becomes available (months 4-30)
- identify recurring alterations that occur in PIN and are also present in early invasive carcinomas (12-30)

Accomplishment: A database of recurrent genetic changes found in pre-cancerous and cancerous specimens has been established. Information such as pathological grades, frequency of occurrence and DNA sequence are included. Chromosomal location of the genetic alterations are summarized (see Aim 3 in Section 2 above)'

Task 4. **To assign recurring alterations to specific chromosomal regions (months 12-36)**

- clone and sequence selected recurring alterations (months 12-36)
  - Year 1: 10 alterations
  - Year 2: 20 alterations
  - Year 3: 20 alterations
- map sequences to chromosomal locations
  - locate alterations by comparing sequences against available human genome sequence (months 12-36)
  - locate remaining alterations by hybridization to human BAC library (months 24-36)

Accomplishment: Alterations have been mapped to the following chromosomal regions. 2p23.1, 8q22.3 and 18q21.31 were unique to PIN while changes at 3p26.3, 4q35.2, , 8q24.13, 11p13, and 16q12.2 were detected only in cancer cells; changes at 1p32.1, 1q44, 5q23.2, 9q22.32 and at 12p12.1 were found in both stages.

Task 5. **To identify candidate genes in the mapped regions for future mutation scanning (months 24-36)**

- search cDNA, EST, microarray and SAGE data bases (months 24-36)

Accomplishment: Task 5 was schedule for Year 3. As indicated in Aim 5 above, "our studies identified several altered chromosomal regions of which includes 1p/q, 4q, 5q, 8q22/24, 10q, 11p 16q and 18q that have been previously reported in the literature. More importantly, novel sites which mapped to chromosomes 2p23.1, 3p26.3, 3q22.3, 4q22.3, 5q23.2, 9q21.3, 9q22.32, 12p12.1, 13q12.2 and 16q12.2 were also found. Mapping these regions within a 2Mbp range was undertaken and some interesting genes were identified. For example, 3p26 harbors a major CaP predisposition locus, those that mapped to 1q44 and 1p13 contain oncogenes, *AKT3* and *LMO2*, respectively, while an alteration that mapped to 4q35.21 contains the *FAT* tumour suppressor gene."

Task 6. **To begin validation of the best 2 candidate genes. (months 18-36)**

- dissection of frozen tissue sections from prostate biopsies
- isolate RNA samples (months 18-36)
- mutation detection (months 24-36)

Accomplishment: Task 6 was initiated in the middle of Year 2 of this project. Frozen prostate biopsies are being collected. RNA extraction procedure has been optimized.

The validation of selected genes was initiated at the end of Year 3. A follow up study after the end of project validated the significance of LMO2 expression in the progression of prostate cancer (Ma et al 2007).

5. Conclusions

As mentioned in the previous annual reports, the work accomplished matched that of the proposed *Tasks* in the *Statement of Work* and the original proposal. The meeting of the milestones suggests that the required materials, infrastructure and expertise are available and capable of support the work proposed.

The following processes have been established: tissue specimen procurement, histopathological evaluation, laser capture microdissection, DNA extraction and quantitation, SMAL-PCR fingerprint comparison, cloning and sequencing, chromosomal localization, databases and bioinformatics.

Scientifically, our results not only demonstrate the feasibility of the approach used in this project, but have also yielded novel recurring genetic alterations in microdissected PINs and invasive carcinoma of the prostate, despite the minute size of such samples.

In addition to the completion of the proposed tasks, the success of this "New Investigator Award" in 2001-2004 has also laid the foundation for initiating additional projects. The establishment of LCM cell capture methodology has enabled spin off projects (and funding from additional sources) yielding gene expression profiles and proteomic data. This in turns led to the discovery of the involvement of growth differentiation factor 15 in prostate carcinogenesis (Cheung et al 2004, Noorali et al 2007). Follow up study on one of the candidate genes in the significance of LMO2 expression in the progression of prostate cancer (Ma et al 2007).

As anticipated in the original proposal, by the end of the project, we have identified a set of genetic loci by virtue of their frequency of alteration in premalignant lesions and subsequently in low grade tumors. We have established a "genetic alterations in prostate cancer" database which catalogs somatic changes present in the various stages of cancer progression. The data was presented at international conferences and the database was publicly accessible on the BC Cancer Research Center web site. These data are extremely valuable in identifying targets for early diagnosis and treatment. Future studies will allow cross-referencing of stage-specific genetic signatures against clinical outcome and thus will allow relating genotype to disease risk and behavior.

6. References

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7. Appendices

None – figures and diagrams are embedded in the body of text.